THE SYNTHESIS OF ACETAMIDO-DEOXY KETOSES BY ACETOBACTER SUBOXYDANS

PART II[1]

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ABSTRACT

The microbiological oxidation of 1-deoxy-1-*N*-methylacetamido-D-glucitol and 2-acetamido-1,2-dideoxy-D-glucitol yielded a syrupy ketose and a crystalline ketose respectively. Crystalline derivatives of each were prepared and structural investigations of the ketoses showed the former to be 6-deoxy-6-*N*-methylacetamido-L-xylohexulose and the latter to be 5-acetamido-5,6-dideoxy-L-xylohexulose.

INTRODUCTION

The microbiological oxidation of unsubstituted polyhydric alcohols has been studied extensively (1, 2, 3, 4, 5, 6), and the oxidation of terminal-substituted polyhydric alcohols has also been investigated in several laboratories (7, 8, 9, 10). Recently we reported the oxidation of the substituted hexitol 2-acetamido-2-deoxy-D-glucitol by *Acetobacter suboxydans* to 5-acetamido-5-deoxy-L-xylohexulose (11) and in this paper we wish to report the microbiological oxidation of 1-deoxy-1-*N*-methylacetamido-D-glucitol (I) to 6-deoxy-6-*N*-methylacetamido-L-xylohexulose (II), and of 2-acetamido-1,2-dideoxy-D-glucitol (III) to 5-acetamido-5,6-dideoxy-L-xylohexulose (IV).

The oxidation of 1-deoxy-1-*N*-methylacetamido-D-glucitol (I) yielded a syrup which gave absorptions in the infrared corresponding to OH, CH, and C=O of the tertiary amide group. A weak band at 1730 cm^{-1} was also observed and was probably due to the presence of a small amount ($\sim$5%) of the acyclic form of the ketose. The results of periodate oxidation indicated that the main bulk of the material existed in the furanose ring form (II). Further evidence for a furanose ring form was given by the ready and rapid formation of a crystalline methyl glycoside (V) which was stable towards alkali but which was readily hydrolyzed with dilute acid. The rate of periodate oxidation of the glycoside (V) indicated that the two free hydroxyl groups, between which cleavage had occurred, were held in the trans configuration by the near-planar furanose ring.

The ketose (II) gave a crystalline phenylosazone (VI) which, when oxidized with periodate by the method of Hough, Powell, and Woods (12), consumed 2 moles of periodate, releasing 0.58 mole formic acid and no formaldehyde. The apparent low formic acid release agreed with the findings of Hough, Powell, and Woods, who obtained similar results from the periodate oxidation of monosaccharide and disaccharide phenylosazones (12). An immediate precipitate of the 1,2-bisphenylhydrazone of mesoxalaldehyde (VII) was obtained from the oxidation. The oxidation results indicated that the osazone (VI) possessed free hydroxyl groups at carbons 3, 4, and 5, and that the ketose (II) from which it was derived was a 2-ketose.

The ketose (II) was very slowly reduced by sodium borohydride to give a syrup which could not be crystallized. This product apparently consisted of the two expected isomeric hexitols although they could not be separated by paper chromatography in several solvent systems.

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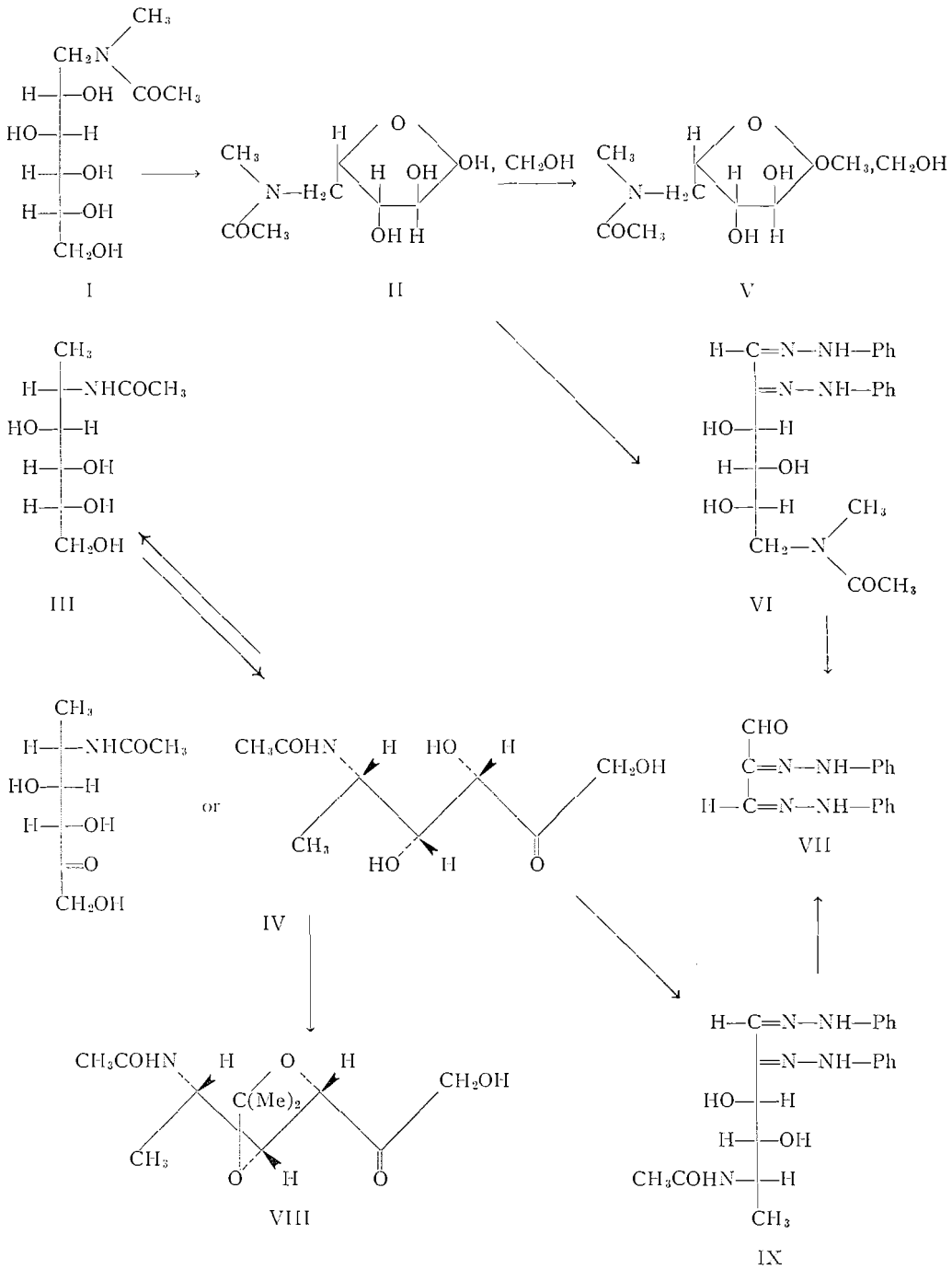
The above results showed that the ketose had the indicated structure (II), the major portion being in the furanose ring form, and that the site of biological oxidation in 1-deoxy-1-*N*-methylacetamido-D-glucitol was at carbon 5, in accordance with the well-known enzyme specificity for oxidations at pH 5–6.5.



The oxidation of 2-acetamido-1,2-dideoxy-D-glucitol (III) by *Acetobacter suboxydans* gave crystalline 5-acetamido-5,6-dideoxy-L-xylohexulose (IV). The results of periodate oxidation indicated that enzymic oxidation had not occurred at the primary alcohol group of the hexitol. The formation of aldofuranose rings was thus excluded on this evidence and the ketose therefore probably existed in the acyclic zigzag form (IV). Evidence to support this conclusion was given by (*a*) the strong, sharp peak at 1735 cm^{-1} which was observed in the carbonyl region of the infrared spectrum, and (*b*) by the fact that the ketose (IV) was not oxidized by bromine water or alkaline hypoiodite solution.

The ketose (IV) rapidly reduced Fehling's solution in the cold and epimerized easily when warmed in pyridine solution or allowed to stand in contact with base for a short time, due to the ease of keto-enol tautomerism in the acyclic form.

With acidified acetone the ketose (IV) gave a syrupy isopropylidene derivative (VIII), the infrared spectrum and periodate oxidation of which indicated that acetal formation had occurred across the hydroxyl groups on carbons 3 and 4, which were therefore favorably situated for this reaction.

Reaction of the ketose (IV) with phenylhydrazine gave a crystalline phenylosazone (IX), which, on periodate oxidation, consumed 1 mole of periodate and released no formic acid or formaldehyde. An immediate precipitate of the 1,2-bisphenylhydrazone of mesoxalaldehyde (VII) was obtained. The above evidence indicated that the phenylosazone (IX) possessed free hydroxyl groups on carbons 3 and 4 and that the ketose (IV) from which it was derived was a 2-ketose.

Reduction of the ketose (IV) with sodium borohydride gave crystalline 2-acetamido-1,2-dideoxy-D-glucitol (III), which confirmed that the hydroxyl groups at carbons 3 and 4 of the ketose (IV) were in the D-*threo* configuration since the position of the keto group had been fixed at carbon 2 by previous evidence.

The above results indicated that the biological oxidation product was the acyclic 2-hexulose, 5-acetamido-5,6-dideoxy-L-xylohexulose (IV), as would be predicted from the Bertrand–Hudson rule for enzymic oxidation by *Acetobacter suboxydans* (13, 14).

EXPERIMENTAL

Melting points are uncorrected and were determined on a Fisher-John melting point apparatus. All evaporations were carried out under reduced pressure at 50° C or less. Optical rotations were measured at 23±3° C in water unless otherwise stated. Paper chromatography was carried out by the descending method (15) using Whatman No. 1 paper in the following solvent systems (v/v):

(A) butan-1-ol/ethanol/water, 9:3:3;
(B) butan-1-ol/pyridine/water, 5:3:2;
(C) ethyl acetate/acetic acid/formic acid/water, 18:3:1:4;
(D) 2-butanone/glacial acetic acid/saturated aqueous boric acid, 9:1:1.

The rates of movement of compounds on paper chromatograms are given relative to that of rhamnose (R_{rh} value). Ketose sugars were detected on paper chromatograms with the orcinol – trichloroacetic acid spray reagent (16), other reducing compounds with the *p*-anisidine hydrochloride spray reagent (17), and non-reducing compounds with the alkaline silver nitrate spray reagent (18). Infrared spectra were measured in chloroform solution or as a dispersion in a potassium bromide pellet, using a Perkin–Elmer Model 21 spectrophotometer. Formaldehyde produced in periodate oxidations was determined by the chromotropic acid method (19).

Section A. Studies on 6-Deoxy-6-*N*-methylacetamido-L-xylohexulose

Preparation of 1-Deoxy-1-N-methylacetamido-D-glucitol

1-Deoxy-1-*N*-methylamino-D-glucitol (Aldrich Chem. Co. sample) was twice recrystallized from ethanol containing a little water to give a creamy-white product which had m.p. 128–129° C, $[\alpha]_D$ −14° (lit. values, m.p. 126° C, $[\alpha]_D$ −18.5±1° (20)).

1-Deoxy-1-*N*-methylamino-D-glucitol was *N*-acetylated in aqueous solution, using the method of Levvy and McAllan (21), to give a yellow syrup which slowly crystallized on desiccation. The product was recrystallized from methanol/ether, then from ethanol/ether to give white crystals which had m.p. 119–121° C, $[\alpha]_D$ −22°.

6-Deoxy-6-N-methylacetamido-L-xylohexulose

1-Deoxy-1-*N*-methylacetamido-D-glucitol (7 g), sorbitol (3 g), yeast extract powder (0.5 g), and potassium dihydrogen phosphate (0.05 g) were dissolved in tap water (140 ml), and the solution was distributed among six 250-ml conical flasks and autoclaved at 15 p.s.i. for 20 minutes. After being cooled, the sterile broths were inoculated with several drops of a 48-hour culture of *Acetobacter suboxydans* grown in sorbitol solution. The flasks were stored in the dark at room temperature and samples were removed at intervals, under sterile conditions, for examination by paper chromatography and estimation of copper-reducing value by the Somogyi method (22). The appearance of L-sorbose (R_{rh} 0.52, solvent A) was noted, and of a spot (R_{rh} 1.1, solvent A) which gave a pink color changing to green with the orcinol – trichloroacetic acid spray reagent. The results of the Somogyi estimations are shown in Table I.

TABLE I

Oxidation of 1-deoxy-1-*N*-methylacetamido-D-glucitol by *Acetobacter suboxydans*

Time (days)	% yield of ketose (corrected for the presence of L-sorbose)
6	7.2
12	23.6
19	35.6

Growth was terminated after 22 days by pouring the broths into two volumes of ethanol. The solution was filtered, deionized by passage through Amberlite I.R. 120 (H^+) and Duolite A4 (OH') resins, and concentrated to a syrup. L-Sorbose crystallized out and was removed and the residual syrup was then separated into three components by chromatography on a cellulose column using butan-1-ol half-saturated with water as the irrigant. The three components were L-sorbose, 1-deoxy-1-*N*-methylacetamido-D-glucitol, and 6-deoxy-6-*N*-methylacetamido-L-xylohexulose. The latter was obtained as a clear, light yellow syrup (2.2 g), $[\alpha]_D$ −57°. An infrared spectrum was obtained by smearing a little of the syrup onto the surface of a potassium bromide pellet, and showed the following main absorptions: OH (3410 cm^{-1}, strong), CH (2960 cm^{-1}, strong), saturated ketone C=O (1730 cm^{-1}, weak), tertiary amide C=O (1625 cm^{-1}, strong). The complete spectrum is given in Appendix A.

Periodate Oxidation of 6-Deoxy-6-N-methylacetamido-L-xylohexulose

An aqueous solution of 6-deoxy-6-*N*-methylacetamido-L-xylohexulose was oxidized under unbuffered conditions using a twofold excess of sodium metaperiodate. The results of the oxidation are shown in Table II.

TABLE II

Periodate oxidation of 6-deoxy-6-*N*-methylacetamido-L-xylohexulose

Time (hours)	Periodate uptake (moles/mole)	Formic acid (moles/mole)	Formaldehyde (moles/mole)
0.08	1.84	1.06	
0.17			0.05
1		1.11	
2			0.10
4	2.05	1.20	
4.5			0.23
6	2.14	1.26	
23			0.26
26	2.44	1.50	

Methyl 6-Deoxy-6-N-methylacetamido-L-xylohexulofuranoside

6-Deoxy-6-*N*-methylacetamido-L-xylohexulose (200 mg) was dissolved in anhydrous methanol (5 ml), and anhydrous methanolic hydrogen chloride (1%, 8 ml) added. The solution was allowed to stand at room temperature for $3\frac{1}{2}$ hours, then neutralized with silver carbonate, filtered, and evaporated to dryness. The resulting pale yellow syrup gave a bright yellow spot on paper chromatograms (R_{rh} 1.57, solvent A) with the *p*-anisidine hydrochloride spray reagent. The syrup gave only a faint spot due to unreacted 6-deoxy-6-*N*-methylacetamido-L-xylohexulose with the alkaline silver nitrate spray reagent. After desiccation for 3 days the syrup crystallized and crystallization was speeded by the addition of methanol/ethyl acetate. The crystals were filtered off, dried, and recrystallized twice from methanol/ethyl acetate to give white needles (50 mg) which had m.p. 109–110° C, $[\alpha]_D$ −97°. The glycoside gave absorptions in the infrared corresponding to OH (3480 cm^{-1}), CH (2940, 2880 cm^{-1}), and tertiary amide C=O (1600 cm^{-1}). The complete spectrum is given in Appendix B. Anal. Calc. for $C_{10}H_{19}O_6N$: C, 48.2%; H, 7.6%; N, 5.6%. Found: C, 48.1%; H, 7.4%; N, 5.7%.

Periodate Oxidation of Methyl 6-Deoxy-6-N-methylacetamido-L-xylohexulofuranoside

The glycoside was oxidized with a twofold excess of sodium metaperiodate in unbuffered aqueous solution. The results of the oxidation are given in Table III. No formaldehyde was detected in the oxidation mixture.

TABLE III

Periodate oxidation of methyl 6-deoxy-6-*N*-methylacetamido-L-xylohexulofuranoside

Time (hours)	Periodate uptake (moles/mole)	Formic acid (moles/mole)
0.08	0.07	0.00
5	0.88	0.03
9	0.98	0.00
23.5	1.00	0.02

6-Deoxy-6-N-methylacetamido-L-xylohexulose Phenylosazone

The osazone was prepared by the usual method using freshly distilled phenylhydrazine and glacial acetic acid. It was recrystallized twice from ethanol/ethyl acetate and once from ethanol/ether to give a bright yellow powder, m.p. 182–183° C. The osazone gave absorptions in the infrared corresponding to OH (3430 cm^{-1}), NH (3270 cm^{-1}), aromatic

CH (3080 cm^{-1}), aliphatic CH (2940, 2880 cm^{-1}), tertiary amide C=O (1625 cm^{-1}), and the aromatic ring (1610, 1500, 750, 690 cm^{-1}). The complete spectrum is given in Appendix C. Anal. Calc. for $C_{21}H_{27}O_4N_5$: C, 61.0%; H, 6.5%; N, 16.9%. Found: C, 60.5%; H, 6.6%; N, 16.2%.

Periodate Oxidation of 6-Deoxy-6-N-methylacetamido-L-xylohexulose Phenylosazone

The osazone was oxidized in 50% aqueous ethanol by the method of Hough, Powell, and Woods (12). The results of the oxidation are shown in Table IV.

TABLE IV

Periodate oxidation of 6-deoxy-6-*N*-methylacetamido-L-xylohexulose phenylosazone

Time (hours)	Periodate uptake (moles/mole)	Formic acid (moles/mole)	Formaldehyde (moles/mole)
0.42	1.90	0.41	0.00
1.66	2.05	0.59	0.00
3.08	2.14	0.58	0.00

1,2-Bisphenylhydrazone of Mesoxalaldehyde (23)

In the periodate oxidation of 6-deoxy-6-*N*-methylacetamido-L-xylohexulose phenylosazone, a curdy mass of orange-yellow needles was precipitated 30 seconds after oxidation had started. The crystals of the 1,2-bisphenylhydrazone of mesoxalaldehyde were centrifuged off and the supernatant returned to the flask for oxidation studies. The crystals were washed with water, dried, and recrystallized from 50% aqueous ethanol, m.p. 189–191° C. An authentic specimen had m.p. 193–194° C and the mixed m.p. was 189–191° C. The infrared spectra of the authentic and derived specimens were identical over the range 4000–600 cm^{-1}. The spectra were given in a previous publication (11).

Sodium Borohydride Reduction of 6-Deoxy-6-N-methylacetamido-L-xylohexulose

An aqueous solution of 6-deoxy-6-*N*-methylacetamido-L-xylohexulose (100 mg) was reduced with an equal weight of sodium borohydride at 5° C for 18 hours. After removal of sodium borohydride and sodium borate the product was obtained as a syrup (100 mg) which gave two spots, R_{rh} 1.1 (unreduced ketose) and R_{rh} 0.61 (polyhydric alcohols) in solvent A, with the alkaline silver nitrate spray reagent. The polyhydric alcohols were separated from the ketose on Whatman 3 MM paper and obtained as a syrup (75 mg) which gave one spot with the alkaline silver nitrate spray reagent on chromatography in solvents A, B, C, and D. The syrup gave one fraction only, when passed through a column of Dowex 50 W resin (8% cross-linked with divinylbenzene, barium salt form, 200–400 mesh).

The syrup had $[\alpha]_D$ −17° ($[\alpha]_D$ of 1-deoxy-1-*N*-methylacetamido-D-glucitol was −22°) and it was concluded that it contained 1-deoxy-1-*N*-methylacetamido-D-glucitol and probably the isomeric L-iditol derivative. Certainly some 1-deoxy-1-*N*-methylacetamido-D-glucitol was present since oxidation of the syrupy mixture by *Acetobacter suboxydans* gave some 6-deoxy-6-*N*-methylacetamido-L-xylohexulose.

Section B. Studies on 5-Acetamido-5,6-dideoxy-L-xylohexulose

Preparation of 2-Acetamido-1,2-dideoxy-D-glucitol

2-Amino-2-deoxy-D-glucose Diethyl Dithioacetal

2-Amino-2-deoxy-D-glucose hydrochloride (15 g), fuming hydrochloric acid (120 ml), and ethanethiol (45 ml) were shaken in a pressure bottle at room temperature for 20 hours.

The solution was diluted with ethanol, lead acetate was added, and then lead carbonate was added to neutrality. The solution was filtered and the lead salts were washed with ethanol. The filtrate and washings were combined and saturated with hydrogen sulphide, a little charcoal was added, and the solution was filtered and concentrated to a yellow syrup. The syrup was dissolved in water and passed through Duolite A4(OH′) resin. The eluate was concentrated to give crystalline 2-amino-2-deoxy-D-glucose diethyl dithioacetal (10.9 g), m.p. 107–109° C, $[\alpha]_D$ −22° (lit. values, m.p. 109–110° C, $[\alpha]_D$ −24° (24)).

*2-Amino-2-deoxy-*D*-glucose Diethyl Dithioacetal Penta-acetate*

2-Amino-2-deoxy-D-glucose diethyl dithioacetal (10 g) was acetylated with acetic anhydride in pyridine solution at room temperature for 24 hours to give 2-amino-2-deoxy-D-glucose diethyl dithioacetal penta-acetate as a colorless syrup (17 g), $[\alpha]_D$ +2° (*c*, 1.54 in chloroform) (lit. value $[\alpha]_D$ +1° (chloroform) (24)).

*2-Acetamido-1,2-dideoxy-*D*-glucitol*

2-Amino-2-deoxy-D-glucose diethyl dithioacetal penta-acetate (17 g) was reductively desulphurized by being boiled under reflux with Raney nickel catalyst for 5 hours in ethanol solution. The solution was filtered and the catalyst was washed several times with hot ethanol. The filtrate and washings were combined and evaporated to dryness, giving 2-amino-1,2-dideoxy-D-glucitol penta-acetate as a semicrystalline syrup (8.6 g). The syrup was dissolved in methanol (400 ml) and cooled to 0° C. A stream of anhydrous ammonia was passed into the solution for 20 minutes and the solution allowed to stand for 2 hours at room temperature, then evaporated to dryness. The crystalline residue was dissolved in methanol and an equal volume of chloroform was added, followed by ether to incipient opalescence. Small needles of 2-acetamido-1,2-dideoxy-D-glucitol crystallized out and were recrystallized by the same method to give a chromatographically pure product (3.9 g) which had m.p. 160–162° C, $[\alpha]_D$ −14° (lit. values, m.p. 162–164° C, $[\alpha]_D$ −9° (25)).

*5-Acetamido-5,6-dideoxy-*L*-xylohexulose*

2-Acetamido-1,2-dideoxy-D-glucitol (3 g), sorbitol (3 g), yeast extract powder (0.5 g), and potassium dihydrogen phosphate (0.05 g) were dissolved in tap water (100 ml). The solution was distributed among five 250-ml conical flasks and autoclaved at 15 p.s.i. for 30 minutes. After being cooled to room temperature, the broths were inoculated with several drops of a 48-hour culture of *Acetobacter suboxydans* grown in sorbitol solution, and stored in the dark at room temperature. Samples were removed at intervals under sterile conditions for chromatographic examination and estimation of copper-reducing values by the Somogyi method (23).

The appearance of L-sorbose (R_{rh} 0.49, solvent A), and a spot (R_{rh} 1.13, solvent A) which gave a yellow color with the orcinol – trichloroacetic acid spray reagent was noted. The results of the Somogyi estimations showed a 16.7% yield of 5-acetamido-5,6-dideoxy-L-xylohexulose after 7 days and a 69% yield after 20 days. Growth was terminated after 31 days by pouring the broths into 2 volumes of ethanol. The solution was filtered and rapidly passed through small beds of Amberlite I.R. 120 (H^+) and Duolite A4 (OH^-) resins at 5° C. The eluate and washings were concentrated at 35–40° C to a brown syrup which slowly crystallized on the addition of ethanol. The crystalline L-sorbose was removed and the residual syrup was fractionated on a cellulose column using butan-1-ol half-saturated with water as the irrigant. Three fractions were obtained: 5-acetamido-5,6-dideoxy-L-xylohexulose, 2-acetamido-1,2-dideoxy-D-glucitol, and L-sorbose.

The 5-acetamido-5,6-dideoxy-L-xylohexulose was obtained as a clear brown syrup (2.1 g), $[\alpha]_D$ −38°. The syrup crystallized after several months but unfortunately only after all the structural work had been completed on the syrup. The crystals were recrystallized from ethanol/ether as rosettes of needles (15 mg) and due to the small amount, a completely pure specimen could not be obtained. The crystals had m.p. 91–93° C, $[\alpha]_D$ −40°.

Anal. Calc. for $C_8H_{15}O_5N$: C, 46.8%; H, 7.3%; N, 6.8%. Found: C, 46.0%; H, 7.2%; N, 6.7%.

The crystals gave absorptions in the infrared corresponding to OH (3400 cm^{-1}), NH (3280 cm^{-1}), CH (2960, 2900 cm^{-1}), saturated ketone C=O (1735 cm^{-1}), amide I (1645 cm^{-1}), amide II (1575 cm^{-1}). The complete spectrum is given in Appendix D.

5-Acetamido-5,6-dideoxy-L-xylohexulose rapidly reduced Fehling's solution in the cold. It was not affected by bromine water at room temperature for 3 hours since no other spots could be detected on paper chromatograms run in solvent A, after bromine water treatment.

Periodate Oxidation of 5-Acetamido-5,6-dideoxy-L-xylohexulose

The ketose was oxidized with sodium metaperiodate at pH 7.5, and under unbuffered conditions and the liberated formaldehyde determined. The results are shown in Table V.

TABLE V

Formaldehyde estimations in the periodate oxidation of 5-acetamido-5,6-dideoxy-L-xylohexulose

	Formaldehyde (moles/mole)	
Time (hours)	pH 7.5	pH 3–4 (unbuffered)
0.17	0.97	0.43
1.17		0.43
4.4		0.41
5	0.89	
9.7		0.39
19	0.76	

Alkaline Hypoiodite Oxidation of 5-Acetamido-5,6-dideoxy-L-xylohexulose

5-Acetamido-5,6-dideoxy-L-xylohexulose was oxidized with alkaline hypoiodite solution by the standard method (26). The results indicated that the 5-acetamido-5,6-dideoxy-L-xylohexulose contained 10% aldose. However, it was found that the pH 11.4 buffer used in the oxidation caused epimerization of the ketose to aldoses which were detected as two new spots on paper chromatograms, R_{rh} 1.6 and R_{rh} 1.8 in solvent A, with the alkaline silver nitrate and *p*-anisidine hydrochloride spray reagents. A similar pattern of spots was obtained when 5-acetamido-5,6-dideoxy-L-xylohexulose was heated at 100° C for 3 hours in pyridine solution. From these results it was concluded that the value of 10% aldose content resulted from epimerization and that the 5-acetamido-5,6-dideoxy-L-xylohexulose contained very little, if any, aldose.

5-Acetamido-5,6-dideoxy-3,4-O-isopropylidene-L-xylohexulose

5-Acetamido-5,6-dideoxy-L-xylohexulose (200 mg) was dissolved in acetone (100 ml) containing concentrated sulphuric acid (4 drops) and shaken for 24 hours at room temperature. The solution was neutralized with barium carbonate, filtered, and evaporated to dryness and the resulting syrup was examined on paper chromatograms run in

solvent A. Two spots were observed with the alkaline silver nitrate spray, R_{rh} 1.2 (5-acetamido-5,6-dideoxy-L-xylohexulose) and R_{rh} 2.5 (5-acetamido-5,6-dideoxy-3,4-*O*-isopropylidene-L-xylohexulose). The isopropylidene derivative was separated by chromatography in solvent A on Whatman 3 MM paper and obtained as a syrup (74 mg), $[\alpha]_D$ −19° (*c*, 1.16 in ethanol). The syrup rapidly reduced Fehling's solution in the cold and gave absorptions in the infrared corresponding to OH (3500 cm^{-1}), CH (3010, 2980, 2910 cm^{-1}), saturated ketone C=O (1730 cm^{-1}), amide I (1670 cm^{-1}), and amide II (1525 cm^{-1}). The complete spectrum is given in Appendix E.

*Periodate Oxidation of 5-Acetamido-5,6-dideoxy-3,4-O-isopropylidene-*L*-xylohexulose*

The isopropylidene ketose was oxidized with a twofold excess of sodium metaperiodate in 50% aqueous ethanol and the liberated formaldehyde determined. The results are shown in Table VI.

TABLE VI

Periodate oxidation of 5-acetamido-5,6-dideoxy-3,4-*O*-isopropylidene-L-xylohexulose

Time (hours)	Formaldehyde (moles/mole)
0.17	0.07
1.17	0.27
4.4	0.29
9.7	0.31

*5-Acetamido-5,6-dideoxy-*L*-xylohexulose Phenylosazone*

5-Acetamido-5,6-dideoxy-L-xylohexulose (200 mg) was treated with freshly distilled phenylhydrazine and glacial acetic acid by the usual method to give a fine yellow powder (110 mg). The osazone was recrystallized from ethanol and had m.p. 156–158° C (decomposes). It gave absorptions in the infrared corresponding to OH (3460 cm^{-1}), NH (3370 cm^{-1}), aromatic CH (3080 cm^{-1}), aliphatic CH (3000, 2910, 2860 cm^{-1}), amide I (1655 cm^{-1}), amide II (1560 cm^{-1}), and the aromatic ring (1610, 1500, 745, 690 cm^{-1}). The complete spectrum is given in Appendix F. Anal. Calc. for $C_{20}H_{25}O_3N_5$: C, 62.7%; H, 6.5%; N, 18.3%. Found: C, 62.85%; H, 6.6%; N, 18.1%.

*Periodate Oxidation of 5-Acetamido-5,6-dideoxy-*L*-xylohexulose Phenylosazone*

The osazone was oxidized with sodium metaperiodate in 50% aqueous ethanol using the method of Hough, Powell, and Woods (12). The results of the oxidation are shown in Table VII.

TABLE VII

Periodate oxidation of 5-acetamido-5,6-dideoxy-L-xylohexulose phenylosazone

Time (hours)	Periodate uptake (moles/mole)
0.42	1.02
1.75	0.98
4.0	1.07

No formic acid or formaldehyde were detected in the oxidation mixture.

During the oxidation a bright orange-yellow precipitate of the 1,2-bisphenylhydrazone of mesoxalaldehyde (23) was obtained. Its identity was proved by melting point, mixed melting point, and identical infrared spectrum with an authentic specimen as described previously in Section A of this publication.

Sodium Borohydride Reduction of 5-Acetamido-5,6-dideoxy-L-xylohexulose

5-Acetamido-5,6-dideoxy-L-xylohexulose (115 mg) was reduced in aqueous solution with an equal weight of sodium borohydride at 0° C for 3 hours. Excess sodium borohydride was converted to sodium borate which was removed as methyl borate after passage through Amberlite I.R. 120 (H^+) resin. The product was obtained as a clear colorless syrup (90 mg). The syrup was dissolved in methanol, an equal volume of chloroform was added, then ether to incipient opalescence, and the solution was stored at 0° C. The resulting crystals were filtered off, washed with methanol/ether, and dried (21 mg). A second crop of crystals could not be obtained from the mother liquors. The crystals had m.p. 163–164° C and an authentic specimen of 2-acetamido-1,2-dideoxy-D-glucitol had m.p. 160–162° C. The mixed m.p. was 157–159° C. The crystals (and mother liquors) gave one spot on paper chromatograms with the alkaline silver nitrate spray reagent. The infrared spectra of the crystals and the authentic specimen of 2-acetamido-1,2-dideoxy-D-glucitol were identical over the range 4000–600 cm^{-1}. The crystals had $[\alpha]_D$ −16° and the authentic material had $[\alpha]_D$ −14°.

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APPENDIX

Infrared Spectra

The following abbreviations are used: S = strong, M = medium, W = weak; wave numbers are expressed in cm^{-1}.

(A) 6-Deoxy-6-N-methylacetamido-L-xylohexulose (smeared on surface of KBr pellet) 3410 (S), 2960 (S), 1730 (W), 1625 (S), 1505 (S), 1425 (S), 1375 (M), 1295 (M), 1255 (M), 1135 (M), 1075 (S), 1035 (S), 935 (M), 895 (W), 865 (W), 815 (W), 695 (W).

*(B) Methyl 6-Deoxy-6-N-methylacetamido-*L*-xylohexulofuranoside* (0.8% in KBr)
3400 (S), 2940 (M), 2880 (M), 1600 (S), 1505 (M), 1465 (M), 1435 (S), 1415 (M), 1385 (M), 1340 (M), 1320 (M), 1280 (M), 1245 (M), 1215 (M), 1165 (S), 1135 (M), 1100 (S), 1050 (M), 1025 (S), 985 (M), 935 (M), 890 (M), 845 (M), 805 (M), 780 (W), 700 (M), 670 (M).

*(C) 6-Deoxy-6-N-methylacetamido-*L*-xylohexulose Phenylosazone* (0.8% in KBr)
3430 (S), 3270 (S), 3080 (S), 2940 (M), 2880 (M), 1625 (S), 1610 (S), 1590 (S), 1540 (S), 1515 (S), 1500 (S), 1460 (M), 1425 (S), 1380 (S), 1340 (M), 1315 (S), 1290 (S), 1260 (S), 1175 (S), 1150 (M), 1105 (S), 1060 (S), 1010 (S), 965 (M), 900 (M), 880 (M), 845 (M), 825 (W), 795 (M), 775 (W), 750 (S), 725 (M), 690 (S).

*(D) 5-Acetamido-5,6-dideoxy-*L*-xylohexulose* (0.8% in KBr)
3400 (S), 3280 (S), 3100 (S), 2960 (M), 2900 (M), 1735 (S), 1645 (S), 1575 (S), 1460 (M), 1430 (M), 1385 (M), 1340 (M), 1325 (M), 1285 (M), 1240 (M), 1185 (W), 1155 (W), 1135 (W), 1115 (S), 1100 (S), 1080 (M), 1050 (M), 975 (W), 965 (W), 955 (W), 850 (W), 825 (W), 740 (M), 690 (M), 655 (M).

*(E) 5-Acetamido-5,6-dideoxy-3,4-O-isopropylidene-*L*-xylohexulose* (6% in chloroform)
3500 (W), 3400 (W), 3010 (M), 2980 (W), 2910 (W), 2410 (W), 1730 (M), 1670 (S), 1525 (S), 1460 (M), 1395 (S), 1385 (S), 1335 (M), 1240 (S), 1170 (M), 1085 (M), 1065 (M), 1045 (M), 1015 (M), 930 (W), 875 (M), 795 (M), 720 (M), 660 (W).

*(F) 5-Acetamido-5,6-dideoxy-*L*-xylohexulose Phenylosazone* (0.8% in KBr)
3460 (S), 3370 (S), 3290 (S), 3080 (M), 3000 (M), 2910 (M), 2860 (W), 1935 (W), 1655 (S), 1610 (S), 1585 (S), 1560 (S), 1540 (S), 1520 (S), 1505 (S), 1460 (S), 1427 (S), 1385 (S), 1307 (S), 1290 (S), 1260 (S), 1175 (M), 1160 (M), 1120 (M), 1080 (M), 1045 (S), 1030 (M), 1015 (M), 975 (W), 940 (W), 905 (W), 870 (W), 845 (W), 815 (W), 785 (M), 755 (S), 745 (S), 690 (S).